

Cancer Risk in Men Exposed In Utero to Diethylstilbestrol

William C. Strohsnitter, Kenneth L. Noller, Robert N. Hoover, Stanley J. Robboy, Julie R. Palmer, Linda Titus-Ernstoff, Raymond H. Kaufman, Ervin Adam, Arthur L. Herbst, Elizabeth E. Hatch

Background: An association between prenatal diethylstilbestrol (DES) exposure and cancer in men, especially testicular cancer, has been suspected, but findings from case-control studies have been inconsistent. This study was conducted to investigate the association between prenatal DES exposure and cancer risk in men via prospective follow-up. **Methods:** A total of 3613 men whose prenatal DES exposure status was known were followed from 1978 through 1994. The overall and site-specific cancer incidence rates among the DES-exposed men were compared with those of the unexposed men in the study and with population-based rates. The relative rate (RR) was used to assess the strength of the association between prenatal DES exposure and cancer development. All statistical tests were two-sided. **Results:** Overall cancer rates among DES-exposed men were similar to those among unexposed men (RR = 1.07; 95% confidence interval [CI] = 0.58 to 1.96) and to national rates (RR = 0.99; 95% CI = 0.65 to 1.44). Testicular cancer may be elevated among DES-exposed men, since the RRs for testicular cancer were 3.05 (95% CI = 0.65 to 22.0) times those of unexposed men in the study and 2.04 (95% CI = 0.82 to 4.20) times those of males in the population-based rates. The higher rate of testicular cancer in the DES-exposed men is, however, also compatible with a chance observation. **Conclusions:** To date, men exposed to DES *in utero* do not appear to have an increased risk of most cancers. It remains uncertain, however, whether prenatal DES exposure is associated with testicular cancer. [J Natl Cancer Inst 2001;93:545-51]

In 1971, Herbst et al. (1) reported an association between *in utero* exposure to

diethylstilbestrol (DES) and the subsequent development of clear cell adenocarcinoma of the vagina among female offspring. Although DES-exposed daughters and their mothers have been studied extensively (1-7), only a few studies have addressed the health of DES-exposed sons. These studies have focused on the subsequent development of genitourinary anomalies or neoplasms, and only the development of benign epididymal cysts has been documented consistently in DES-exposed men (3,8-12). Four case-control studies (13-16) have provided evidence of testicular cancer among men prenatally exposed to either DES or estrogen analogues. This association could, however, be due to differential recall of DES exposure by the subjects or their mothers subsequent to a diagnosis of cancer. Furthermore, two other case-control studies (17,18) failed to identify any association between prenatal DES exposure and testicular cancer risk.

DES is a nonsteroidal estrogen, and maternal DES use increases fetal estrogen exposure and, possibly, the risk of testicular cancer as well. Other prenatal or perinatal factors that may reflect estrogen levels during pregnancy (19-22) have also been associated with testicular cancer. Elevated testicular cancer rates have been reported among men who were members of twin pairs (23), whose mothers experienced severe nausea while pregnant with them (24), who had neonatal jaundice (25), who were their mothers' early pregnancies (19,26), who had either low or

Affiliations of authors: W. C. Strohsnitter, K. L. Noller, Department of Obstetrics and Gynecology, Tufts University School of Medicine, Boston, MA; R. N. Hoover, E. E. Hatch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; S. J. Robboy, Department of Pathology, Duke University Medical Center, Durham, NC; J. R. Palmer, Department of Epidemiology and Biostatistics, Slone Epidemiology Unit, Boston University School of Public Health, Brookline, MA; L. Titus-Ernstoff, Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH; R. H. Kaufman (Department of Obstetrics and Gynecology), E. Adam (Department of Epidemiology), Baylor College of Medicine, Houston, TX; A. L. Herbst, Department of Obstetrics and Gynecology, University of Chicago, IL.

Correspondence to: William C. Strohsnitter, M.S., Department of Obstetrics and Gynecology, New England Medical Center, North Bldg., 750 Washington St., Boston, MA 02111 (e-mail: wstrohsnitter@lifespan.org).

See "Notes" following "References."

© Oxford University Press

high birth weight (25,27), who were born prematurely (17), and who were born to older women (28). Men whose mothers experienced pre-eclampsia while pregnant with them experienced decreased cancer rates (25).

The purpose of this study was to determine and to compare rates of testicular and other cancers among DES-exposed and unexposed men who were followed in a prospective study. Elevated testicular cancer rates among DES-exposed men would lend support to the hypothesis that increased fetal estrogen exposure contributes to testicular cancer development. This report describes the results of the first 16 years of follow-up of this cohort.

SUBJECTS AND METHODS

Study Participants

Four different cohorts of men (Mayo Clinic [Rochester, MN], Dieckmann [Chicago, IL], Horne [Boston, MA], and Women's Health Study [WHS] [Boston; Portland, ME; and Hanover, NH]) whose prenatal DES exposure status is known, were included in this study (Table 1). Exposed men in the Mayo Clinic cohort were identified in the late 1970s by a medical record review. This review indicated that, among all women receiving prenatal care at the Mayo Clinic from 1940 to 1960, a total of 813 males were born to women who had taken DES at some time during that pregnancy (29). In 1978, a subset of these exposed men was examined for genitourinary anomalies (8). A sample of 734 men whose medical records contained no evidence of any exogenous hormone exposure during gestation was drawn from the remaining births at the Mayo Clinic and matched to the subset of examined DES-exposed men by birth date, maternal age, birth order, and maternal residence (8). In all, 754 exposed and 724 unexposed men identified from the Mayo Clinic were alive, cancer free, and eligible for follow-up from 1978 through 1994.

The Dieckmann cohort includes more than 800 men whose mothers, while pregnant with them, participated in a trial conducted in the early 1950s to

examine the efficacy of DES in preventing miscarriages (30). From 1974 to 1976, a total of 309 (73%) of the DES-exposed and 302 (71%) of the unexposed men were examined for adverse health outcomes, including genital abnormalities (9). The last contact with this cohort before 1994, the end of the current follow-up, was during a mail questionnaire survey in 1991 (10).

The Horne cohort consists of 400 exposed men and 290 of their unexposed brothers whose mothers were treated during pregnancy by an infertility specialist in the Boston area. The Horne cohort was assembled in the mid-1970s, and exposed and unexposed men were mailed yearly questionnaires through the 1980s. The data from this cohort have not been analyzed previously.

The WHS cohort consists of 477 DES-exposed and 904 unexposed men whose mothers participated in a study to investigate the association between DES use and maternal breast cancer risk (4). Women enrolled in the WHS were contacted for their permission to enroll their sons in the current follow-up study. Heretofore, no follow-up has been conducted on men borne to WHS participants.

Follow-up

The first systematic follow-up of all four cohorts occurred in 1994. Men were identified as potential participants in this study based on either previous follow-up efforts or, in the case of the WHS cohort, tracing efforts through their mothers. Potential participants were then mailed questionnaires requesting information about their health history and cancer risk factors. If the questionnaire was not returned within 3 weeks, another was mailed. If the second questionnaire was not returned, the potential participant was contacted by telephone, and a trained study assistant verbally administered the questionnaire.

Some men ($n = 853$) were not included in the 1994 follow-up. They had died before 1978, the start of the current follow-up ($n = 145$); they could not be located ($n = 178$); they were unwilling to participate during previous follow-ups ($n = 176$); or their mothers denied permission to contact them ($n = 354$) (Table 1). Follow-up of the 48 men who died from 1978 through 1994 was conducted by reviewing their death certificates for reference to cancer development. Medical records and pathology reports were requested from all participants

who reported any cancer diagnosis. The pathologist associated with this study (S. J. Robboy) independently reviewed specimens of all reported germ cell cancers.

All men participating in this follow-up provided informed consent in accordance with the policies of institutional review boards at the National Cancer Institute (Bethesda, MD) and the respective recruiting centers.

Statistical Analysis

Cancer incidence in the DES-exposed men was internally compared with that in the unexposed men and externally compared with national incidence rates. Unexposed men began person-year accrual in this study on January 1, 1978, because they were identified in the late 1970s. Person-time accrual continued until the date of the first cancer diagnosis, the date of the last known follow-up conducted by the individual study centers, or the date of response to the 1994 questionnaire. For the internal comparison, person-year accrual for the DES-exposed men also began in 1978, but because they had follow-up information from birth, their person-year accrual started at birth when cancer incidence was compared externally with national rates.

Information on most cancer covariates was obtained either from the 1994 questionnaire or, for those men who did not respond to the 1994 questionnaire, from previous follow-up data. For the internal comparison of site-specific cancer rates, birth year and age were considered to be potential confounders. For the analysis of total cancer incidence in the internal comparison, educational level and smoking and alcohol habits were also considered to be potential confounders. Additional information that was available only for the men in the Mayo Clinic cohort was included in the analysis of the association between DES exposure and testicular cancer risk within this cohort. This information includes birth weight, pregnancy order, maternal breast cancer history, and history of cryptorchidism, which some studies (14-16,19,25-27) have independently associated with testicular cancer risk. DES was commonly prescribed to prevent threatened miscarriages that were, in some instances, presaged by vaginal bleeding. Vaginal bleeding could possibly indicate, as well, abnormal fetal development such as testicular malformation. Vaginal bleeding was, therefore, controlled in the analysis of DES

Table 1. Follow-up information on diethylstilbestrol (DES)-exposed and unexposed men

	Mayo Clinic cohort		Dieckmann cohort		Women's Health Study cohort		Horne cohort		Total	
	DES exposed	No DES	DES exposed	No DES	DES exposed	No DES	DES exposed	No DES	DES exposed	No DES
Identified men	813	734	425	423	477	904	400	290	2115	2351
Men available for follow-up after 1978*	754	724	309	302	363	671	283	207	1709	1904
Participants†	660	592	205	187	253	441	247	174	1365	1394
Participation rate, %‡	87.5	81.8	66.3	61.9	69.7	65.7	87.3	84.1	79.9	73.2
Overall follow-up rate, %§	81.2	80.7	48.2	44.2	53.0	48.8	61.8	60.0	64.5	59.3

*Availability for follow-up excludes men who died before 1978 and those who could not be contacted (untraceable or their mothers denied permission to contact).

†Participation consisted of either completion of 1994 questionnaire or death certificate review of deaths from 1978 through 1994.

‡Participation rate was calculated by dividing the number of participants by the total number of men available for follow-up after 1978.

§Overall follow-up rate was calculated by dividing the number of participants by the total number of identified men.

exposure and testicular cancer among men in the Mayo Clinic cohort. The effect of gestational age of DES exposure on testicular cancer risk was analyzed by including as exposed only those men whose exposure began in the first trimester of their mothers' pregnancies.

Relative rates (RRs) were used to summarize the comparison of cancer rates between DES-exposed and unexposed men. Standardized incidence ratios (SIRs) summarized the comparison of cancer rates between the DES-exposed men in this study and the national cancer rates. The national cancer rates used in the SIR determinations were based on those obtained from the Connecticut Tumor Registry (before 1970) and the Surveillance, Epidemiology, and End Results¹ (SEER) registry (from 1973 through 1994). The SIRs were adjusted for age and year of birth. RRs, adjusting for all covariates, were determined by Poisson regression modeling (31) by use of SAS Proc GENMOD (32). The exact 95% confidence intervals (CIs) associated with the effect estimates and exact two-tailed *P* values were determined by use of StatXact 4 for Windows (33).

RESULTS

Participation Rates

The total participation rate for all four cohorts combined (including men who responded in 1994 and men who died after 1978) was 76.4% overall—79.9% for the DES-exposed men and 73.2% for the unexposed men. The overall participation rates for the individual cohorts were 85% for the Mayo Clinic, 64% for Dieckmann, 86% for Horne, and 67% for the WHS (Table 1).

Among the four study cohorts, a total of 49 cancer cases (28 in the DES-exposed men and 21 in the unexposed men) were identified by either questionnaire response or death certificate review. Five of the DES-exposed men were diagnosed with cancer before 1978, and the remaining 44 were diagnosed from 1978 to 1994. Of the 49 cases, 38 were reported on the 1994 follow-up survey, and 11 cases, including one of testicular cancer, were detected by reviewing death certificates. Pathology reports were sought for all identified cases and were obtained for all but seven of the cancer cases. Because all but one of the pathology reports obtained confirmed the presence of the reported cancer, we include 48 identified cancers in the tables presenting overall cancer rates.

Covariate Distribution

Among the 2759 men participating in the 1994 follow-up, there were no appreciable differences between the DES-exposed and unexposed men in the total

cohort with respect to length of follow-up, maternal age, mortality after 1978, race, or smoking. More DES-exposed men, however, had completed 4 years of college (60.8% DES-exposed versus 52.3% unexposed men; *P* = .001) and regularly consumed alcoholic beverages (88.2% DES-exposed versus 85.2% unexposed men; *P* = .025) (Table 2). Within the Mayo Clinic cohort, there was no appreciable disparity between the DES-exposed and unexposed men in the percentage of men with a history of cryptorchidism or whose mothers had a history of breast cancer. DES-exposed men in the Mayo Clinic cohort, however, were less likely to weigh more than 4000 g at birth (*P* = .04) and were more likely to be their mother's second or later pregnancy (*P* = .001) and to have mothers who had experienced vaginal bleeding when pregnant with them (*P* = .001).

External Comparison

The overall rate of cancer in DES-exposed men within the entire study population was not increased compared with the national rate among men of similar age and race. This comparison is based on 27 cases of cancer reported for all sites (SIR = 0.99; 95% CI = 0.65 to 1.44; *P* = .94) (Table 3). The rate of cancer in all sites combined among the unexposed men in the study population was also similar to the national rate. In contrast, the testicular cancer rate among the DES-exposed men was higher than the national

rate. This elevation, however, did not achieve statistical significance (SIR = 2.04; 95% CI = 0.82 to 4.20; *P* = .09). When the one case of germ cell tumor of the mediastinum was included in the analysis, the SIR for all germ cell cancers increased and approached statistical significance (SIR = 2.23; 95% CI = 0.96 to 4.40; *P* = .058).

There were nine cases of testicular cancer diagnosed among the men in the four cohorts. Eight of the nine cases of testicular cancer were diagnosed after 1978, when the internal comparison began, and seven of the nine cancers occurred in DES-exposed men. The age range at the time of testicular cancer diagnosis in the DES-exposed men was 23–41 years; the two unexposed men who developed testicular cancer were 28 and 40 years old when they were diagnosed. None of the men in the Mayo Clinic cohort who were diagnosed with testicular cancer had a history of cryptorchidism, low birth weight, or maternal breast cancer. The mother of one man, however, had experienced vaginal bleeding during the index pregnancy. Independent review of the tissue specimens obtained from these men verified the diagnosis of testicular cancer noted in the pathology report. The testicular tumor types among the DES-exposed cases included seminoma (*n* = 2), malignant teratoma (*n* = 1), embryonal carcinoma (*n* = 1), undifferentiated carcinoma (*n* = 1), and combinations of these (*n* = 2).

Table 2. Characteristics of diethylstilbestrol (DES)-exposed and unexposed study participants*

Characteristic	DES exposed (<i>n</i> = 1365)	No DES (<i>n</i> = 1394)	<i>P</i> †
Mean length of follow-up, y	16.9	16.9	.92
Mean age at start of follow-up, y	24.5	24.4	.75
Mean maternal age at index birth, y‡	27.7	27.6	.98
Deceased (%)§	24 (1.8)	24 (1.7)	.94
Race, white (%)	1305 (97.3)	1343 (98.0)	.10
4-y college degree or higher (%)	830 (60.8)	729 (52.3)	.001
Positive maternal breast cancer history (%)‡	90 (6.7)	90 (6.6)	.91
Participant reported cryptorchidism (%)‡	39 (2.9)	27 (2.0)	.11
Participant first birth (%)‡	230 (35.5)	211 (36.3)	.78
Participant second pregnancy or higher (%)‡	505 (79.2)	386 (66.3)	.001
Birth weight >4000 g (%)‡	60 (9.7)	76 (13.5)	.041
Maternal prenatal vaginal bleeding (%)‡	226 (34.3)	54 (9.1)	.001
Ever smoked cigarettes regularly (%)	655 (48.8)	669 (48.8)	.99
Ever drank alcoholic beverages regularly (%)	1183 (88.2)	1167 (85.2)	.025

*Information for most variables was obtained from the 1994 questionnaire; percentages were calculated by use of a denominator that excluded those with missing information for that characteristic.

†Two-sided *P* values were determined by the χ^2 test of independence for binomial variables and by the Student's *t* test for continuous variables.

‡Available for the Mayo Clinic cohort only. Percentage calculated on Mayo Clinic participants in 1994 or those who died after January 1, 1978 (DES exposed [*n* = 660]; unexposed [*n* = 592]) with information available.

§Number known to be deceased after January 1, 1978.

Table 3. Cancer risk in diethylstilbestrol (DES)-exposed and unexposed men compared with population-based rates*

Cancer site or type	DES-exposed men (n = 1787)			Unexposed men (n = 1625)		
	Observed	Expected	SIR (95% CI)	Observed	Expected	SIR (95% CI)
All cancers combined†	27	27.3	0.99 (0.65 to 1.44)	21	20.2	1.05 (0.65 to 1.60)
Digestive system	4	2.7	1.50 (0.40 to 3.84)	0	2.5	0.00 (0.0 to 1.48)
Lung	2	1.4	1.39 (0.17 to 5.00)	2	1.5	1.37 (0.16 to 4.94)
Germ cell	8	3.6	2.23 (0.96 to 4.40)	2	2.7	0.73 (0.08 to 2.63)
Testicular	7	3.4	2.04 (0.82 to 4.20)	2	2.7	0.75 (0.09 to 2.69)
Melanoma	3	3.1	0.98 (0.20 to 2.86)	2	2.7	0.74 (0.19 to 2.97)
Thyroid	2	0.9	2.27 (0.27 to 8.18)	3	0.7	4.39 (0.89 to 12.83)
Bone	2	0.6	3.31 (0.40 to 11.96)	2	0.2	10.08 (1.21 to 36.41)
Lymphatic/hematopoietic	4	7.4	0.54 (0.15 to 1.38)	3	4.4	0.68 (0.14 to 1.99)
Other‡	2	7.6	0.26 (0.03 to 0.95)	7	5.4	1.30 (0.52 to 2.67)
Person-years	65 872§			24 972		

*Rates before 1970 were obtained from the Connecticut Tumor Registry. Rates from 1973 through 1994 are from the Surveillance, Epidemiology, and End Results (SEER) registry. SIR = standardized incidence ratio; CI = confidence interval.

†Includes 41 cancers confirmed by pathology report, medical record, or death certificate and seven cancers that were reported but not confirmed.

‡Other cancer sites include one case each of oral and kidney cancers in the DES-exposed men, three cases of oral cancers, one case of bladder cancer, one case of larynx cancer, one case of soft tissue sarcoma, and one case of cancer of unknown primary site among the unexposed men.

§Person-years for DES-exposed men are from birth until last follow-up date and include follow-up conducted by individual study centers.

||Person-years for unexposed men are from 1978 until last follow-up date and include follow-up conducted by individual study centers.

Internal Comparison

Results of the internal comparison were similar to those for the comparison to the national rates. The unadjusted comparison of total cancer incidence among DES-exposed men with that among unexposed men indicated no association between DES exposure and total cancer risk in the entire cohort (RR = 1.07; 95% CI = 0.58 to 1.96; $P = .88$). When each of the four cohorts was analyzed separately, only the DES-exposed men originating from the Mayo Clinic cohort had more than a twofold increase in overall cancer risk (Table 4). This increased cancer risk was not, however, statistically significant (RR = 2.21; 95% CI = 0.93 to 5.69; $P = .10$).

The testicular cancer rate was also compared between DES-exposed and unexposed men (Table 4). Because the

distribution of potentially confounding factors for which there was complete information from all cohorts was essentially the same for exposed and unexposed men, only the unadjusted RR is presented. The rate of testicular cancer among the DES-exposed men for all four cohorts combined was greater than that among the unexposed men (RR = 3.05; 95% CI = 0.65 to 22.0; $P = .18$). This increase, however, was not statistically significant. Furthermore, it was due solely to the experience of the Mayo Clinic cohort, where the testicular cancer rate was elevated among the DES-exposed men compared with those unexposed. Again, this increase was not statistically significant (RR = 4.53; 95% CI = 0.63 to 107.9; $P = .22$). Although the association between DES exposure and testicular cancer risk was essentially unchanged when controlling for pregnancy order, history of

maternal breast cancer, low birth weight, or cryptorchidism, it increased when controlling for maternal vaginal bleeding during the index pregnancy (RR = 5.29; 95% CI = 0.70 to 128.7; $P = .20$). While it was evident that prenatal DES exposure was not associated with the overall cancer rate, the possible association between this exposure and increased testicular cancer warranted further investigation.

An association between prenatal DES exposure and testicular cancer risk could depend on the level of exposure. Investigation of a dose-response relationship between prenatal DES exposure and testicular cancer risk for the entire study cohort, however, was not possible because the dose of DES prescribed during the index pregnancy was not consistently recorded at all of the study centers. Among those members of the Mayo Clinic cohort with consistent dose recording, however, the

Table 4. Cancer risk in diethylstilbestrol (DES)-exposed men compared with cancer risk in unexposed men by individual cohorts*

Cohort	All cancers combined†				Testicular cancer‡			
	No. of cases				No. of cases			
	DES-exposed men	Unexposed men	RR§	95% CI	DES-exposed men	Unexposed men	RR§	95% CI
All cohorts combined	22	21	1.07	0.58 to 1.96	6	2	3.05	0.65 to 21.96
Mayo Clinic	17	7	2.21	0.93 to 5.69	5	1	4.53	0.63 to 107.9
Dieckmann	1	4	0.24	0.009 to 1.90	0	0	—	—
Horne and Women's Health Study cohort	4	10	0.47	0.13 to 1.46	1	1	1.17	0.03 to 45.76

*Cancer risk comparison is based on data collected from January 1, 1978, through December 31, 1994. RR = relative rate; CI = confidence interval.

†Includes both confirmed and reported cancers.

‡All reported cases of testicular cancer were confirmed by pathology report.

§Person-years for both DES-exposed and unexposed men are computed from January 1, 1978, until last follow-up date. Unadjusted RRs are reported.

||The Horne and Women's Health Study cohorts were analyzed together because of the low number of cancer cases in each cohort.

median DES doses for exposed men who developed testicular cancer and exposed men who did not develop testicular cancer were comparable at 12.5 and 10 mg/day, respectively. In contrast, mothers of the Dieckmann and the Horne cohort participants received considerably higher doses of DES, because the medical centers contributing to those cohorts adhered to the Smith and Smith regimen (34), which recommended a total dose of 12 g for the entire pregnancy. Thus, these data suggest that prenatal DES exposure at low doses may be associated with testicular cancer risk.

The gestational timing of DES exposure and early progestin exposure were analyzed as possible factors that might also influence testicular cancer risk. Only the Mayo Clinic and the Dieckmann cohorts consistently recorded first dates of DES exposure. Within the Mayo Clinic cohort, 388 (62.2%) of 624 DES-exposed men were exposed during the first trimester of pregnancy. When only these 388 men were considered to be DES exposed, the point estimate for the association between DES exposure and testicular cancer risk did not change, but the 95% CI was narrowed (RR = 4.46; 95% CI = 0.79 to 34.82; $P = .08$). The RR remained essentially constant because one testicular cancer case and a proportionate amount of person-time shifted from the exposed to the unexposed category. Within the Dieckmann cohort, where no association between DES exposure and testicular cancer risk was observed, an even higher percentage of men, 79.6% (246 of 309), were exposed to DES in the first trimester of pregnancy. Early progestin exposure could also influence the impact of DES exposure on testicular cancer risk. When men who were exposed to both progestins and DES in the first trimester ($n = 138$) were excluded from the analysis of DES and testicular cancer in the Mayo Clinic cohort, the association increased and achieved statistical significance (RR = 5.91; 95% CI = 1.05 to 46.1; $P = .04$). The above data suggest that early progestin exposure may influence the association between DES exposure and testicular cancer. The effect of gestational timing of exposure on this association is not clear.

DISCUSSION

After 16 years of follow-up of 1365 men exposed and 1394 men not exposed to DES *in utero*, there is no association between prenatal exposure to DES and

overall cancer risk. It is still uncertain as to whether prenatal DES exposure is associated with testicular cancer risk. The increased testicular cancer risk that was identified among DES-exposed men was limited to those men within the Mayo Clinic cohort and could thus be a chance finding. Inclusion of the testicular cancer experience among the other cohorts increased the precision of the effect estimate, but the resultant twofold to threefold increase in testicular cancer incidence still did not achieve statistical significance.

This study has several limitations. There was only a 30% power to detect a statistically significant effect with an RR of 3.21 at the .05 level because of the low frequency of testicular cancer cases and the size of the entire study population. An 80% power to detect the same effect at the .05 level would have required enrollment of 5500 men in each exposure category. It is possible that this study could have incurred a selection bias because approximately 38% of the men who qualified for the study did not participate. Consequently, the association between *in utero* DES exposure and cancer development may have been different if the study had had a higher overall participation rate. The overall cancer rates observed among the unexposed men in this study are similar to national cancer rates, however, and the participation rates for the DES-exposed and unexposed men were also similar. It is, therefore, difficult to determine what impact, if any, nonparticipation had on the study results. While differential exposure misclassification based on disease development is possible, it is not likely, since men were identified as DES exposed before disease follow-up began. Conversely, cases were identified independently of exposure status, since both DES-exposed and unexposed men had similar participation rates and were verified in the same manner. Nonetheless, the possibility cannot be ruled out that increased detection of cancer among the DES-exposed men was because of their more vigilant surveillance.

Results in this study could possibly have been confounded by known and unknown factors. The distribution of most of the known cancer covariates was similar among the DES-exposed and unexposed men for those who provided such information. There was, therefore, little poten-

tial for confounding by these factors. DES was prescribed for threatened miscarriage, which itself could indicate developing testicular malformation. Confounding by this indication for DES is, therefore, possible. Men whose mothers experienced vaginal bleeding while pregnant with them, however, actually had a lower testicular cancer risk than men whose mothers did not experience bleeding. Consequently, the association between DES exposure and testicular cancer increased after controlling for threatened miscarriage.

The findings of this study with respect to testicular cancer are consistent with those from numerous case-control studies (13-16). These retrospective studies were limited, however, by the small number of DES-exposed cases or incomplete exposure verification. Other case-control studies also failed to identify any association between DES exposure and testicular cancer risk. Those studies, however, were conducted within populations of men that had a low prevalence of DES exposure (18) or that had not reached the age at which testicular cancer is commonly diagnosed (17). Moreover, neither of those studies verified DES exposure by medical record review.

Maternal usage of DES, a nonsteroidal estrogen, results in elevated fetal estrogen exposure. Other pregnancy characteristics, such as older maternal age, dizygotic twin membership, birth order, and preeclampsia, have been associated with both pregnancy estrogen levels (19-22) and testicular cancer risk (17,19,23-28). Given what is known about the embryologic development of the male gonad, it is biologically plausible that *in utero* estrogen exposure could affect adult testicular cancer risk. Estrogen receptors have been found in most fetal structural gonadal cells in males, but the levels of those receptors diminish shortly after birth (35,36). These cells include the Sertoli cells, which produce müllerian-inhibiting hormone (MIH), a substance that degrades the müllerian ducts, primitive female gonadal structures present in the young male fetus (37). DES has been shown to reduce MIH activity, resulting in the incomplete breakdown of the embryologic female gonads (38). The persisting müllerian remnants may become cancerous in adult life.

Progesterone decreases the production of estrogen receptors (39-42). Because these receptors may mediate the effect of

estrogenic substances on MIH activity, exposure to exogenous progestins, which are progesterone analogues, might reduce the effect of estrogen exposure on testicular cancer risk. The data in this study lend limited support to this possibility, because excluding men exposed to both DES and progestins from the analysis increased the estimate of effect of prenatal DES exposure on testicular cancer development.

In addition to progestin exposure, levels and timing of DES exposure may affect the association between DES exposure and testicular cancer risk. On average, the men within the Mayo Clinic cohort were exposed to lower DES doses than were the men from the three remaining cohorts. That an association between DES exposure and testicular cancer risk was observed among the cohort with low but not high DES exposures is consistent with the results of an animal study where prostate abnormalities occurred in mice receiving low but not high DES doses (43). The effect of gestational age at first DES exposure on testicular cancer risk cannot be readily explained from the current data. At least 60% of the men enrolled in the Mayo Clinic cohort were first exposed to DES in the first trimester. Paradoxically, more than 80% of the men from the Dieckmann cohort also incurred their first DES exposure in the first trimester, yet no association between DES exposure and testicular cancer risk was observed among these men. Consequently, the current study data do not clarify what influence either timing or extent of exposure has on the association between prenatal DES exposure and testicular cancer risk.

The strengths of this study include the large size of the study population, the certainty of exposure classification because of prenatal medical record review, and the prospective design of the study. To date, the results of this study indicate that *in utero* exposure to DES does not influence the development of most cancers in men. Whether such exposure, however, is associated with testicular cancer risk is still unclear. The association between DES exposure and testicular cancer risk observed in this study could be due to chance. These results are consistent, however, with what is known about the biology of testicular cancer, and they lend support to the hypothesis that the prenatal hormonal environment may influence the development of testicular cancer in adults. None-

theless, it is highly unlikely that DES exposure plays a major role in the increases in testicular cancer rates that have been observed in developed countries over the past 60 years (44). In conclusion, no increase in overall cancer risk was observed among this cohort of DES-exposed men. The men currently being followed are approaching the age at which most cancers are diagnosed. Consequently, further follow-up of this population will enable investigators to determine what cancers, if any, are affected by prenatal DES exposure.

REFERENCES

- (1) Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* 1971;284:878-81.
- (2) Colton T, Greenberg E, Noller K, Resseguie L, Van Bennekum C, Heeren T, et al. Breast cancer in mothers prescribed diethylstilbestrol in pregnancy. Further follow-up. *JAMA* 1993;269:2096-100.
- (3) Bibbo M, Gill WB, Azizi F, Blough R, Fang VS, Rosenfield RL, et al. Follow-up study of male and female offspring of DES-exposed mothers. *Obstet Gynecol* 1977;49:1-8.
- (4) Greenberg ER, Barnes AB, Resseguie L, Barrett JA, Burnside S, Lanza LL, et al. Breast cancer in mothers given diethylstilbestrol in pregnancy. *N Engl J Med* 1984;311:1393-8.
- (5) Labarthe D, Adam E, Noller KL, O'Brien PC, Robboy SJ, Tilley BC, et al. Design and preliminary observations of the National Cooperative Diethylstilbestrol Adenosis (DESAD) Project. *Obstet Gynecol* 1978;51:453-8.
- (6) Vessey M, Fairweather DV, Norman-Smith B, Buckley J. A randomized double-blind controlled trial of the value of stilbestrol therapy in pregnancy: long-term follow-up of mothers and their offspring. *Br J Obstet Gynaecol* 1983;90:1007-17.
- (7) Hatch EE, Palmer JR, Titus-Erstoff L, Noller KL, Kaufman RH, Mittendorf R, et al. Cancer risk in women exposed to diethylstilbestrol *in utero*. *JAMA* 1998;280:630-4.
- (8) Leary FJ, Resseguie LJ, Kurland LT, O'Brien PC, Emslander RF, Noller KL. Males exposed *in utero* to diethylstilbestrol. *JAMA* 1984;252:2984-9.
- (9) Gill WB, Schumacher GF, Bibbo M, Straus FH, Schoenberg HW. Association of diethylstilbestrol exposure *in utero* with cryptorchidism, testicular hypoplasia and semen abnormalities. *J Urol* 1979;122:36-9.
- (10) Wilcox AJ, Baird DD, Weinberg CR, Hornsby PP, Herbst AL. Fertility in men exposed prenatally to diethylstilbestrol. *N Engl J Med* 1995;332:1411-6.
- (11) Niculescu A. Effects of *in utero* exposure to DES on male progeny. *J Obstet Gynecol Neonatal Nurs* 1985;14:468-70.
- (12) Conley GR, Sant GR, Ucci AA, Mitcheson HD. Seminoma and epididymal cysts in a young man with known diethylstilbestrol exposure *in utero*. *JAMA* 1983;249:1325-6.
- (13) Moss A, Osmond D, Bacchetti P, Torti FM, Gurgin V. Hormonal risk factors in testicular cancer: a case-control study. *Am J Epidemiol* 1986;124:39-52.
- (14) Depue RH, Pike MC, Henderson BE. Estrogen exposure during gestation and risk of testicular cancer. *J Natl Cancer Inst* 1983;71:1151-5.
- (15) Henderson BE, Benton B, Jing J, Yu MC, Pike MC. Risk factors for cancer of the testis in young men. *Int J Cancer* 1979;23:598-602.
- (16) Schottenfeld D, Warschauer ME, Sherlock S, Zauber AG, Leder M, Payne R. The epidemiology of testicular cancer in young adults. *Am J Epidemiol* 1980;112:232-46.
- (17) Gershman ST, Stolley PD. A case-control study of testicular cancer using Connecticut tumour registry data. *Int J Epidemiol* 1988;17:738-42.
- (18) Brown LM, Pottern LM, Hoover RN. Prenatal and perinatal risk factors for testicular cancer. *Cancer Res* 1986;46:4812-6.
- (19) Panagiotopoulou K, Katsouyanni K, Petridou E, Garas Y, Tzonou A, Trichopoulos D. Maternal age, parity, and pregnancy estrogens. *Cancer Causes Control* 1990;11:119-24.
- (20) Kappel B, Hansen K, Moller J, Faaborg-Andersen J. Human placental lactogen and dU-estrogen levels in normal twin pregnancies. *Acta Genet Med Gemellol (Roma)* 1985;34:59-65.
- (21) Lipworth L, Trichopoulos D, Petridou E. Maternal pregnancy estrogens, breast cancer risk, and the Utah data [letter]. *J Natl Cancer Inst* 1995;87:144-5.
- (22) Rosing U, Carlstrom K. Serum levels of unconjugated and total oestrogens and dehydroepiandrosterone, progesterone and urinary oestriol excretion in pre-eclampsia. *Gynecol Obstet Invest* 1984;18:199-205.
- (23) Braun MM, Ahlbom A, Floderus B, Brinton LA, Hoover RN. Effect of twinning on incidence of cancer of the testis, breast, and other sites (Sweden). *Cancer Causes Control* 1995;6:519-24.
- (24) Petridou E, Roukas K, Dessypris N, Aravantinos G, Bafaloukos D, Efraimidis A, et al. Baldness and other correlates of sex hormones in relation to testicular cancer. *Int J Cancer* 1997;71:982-5.
- (25) Akre O, Ekblom A, Hsieh CC, Trichopoulos D, Adami HO. Testicular nonseminoma and seminoma in relation to perinatal characteristics. *J Natl Cancer Inst* 1996;88:883-9.
- (26) Prener A, Hsieh CC, Engholm G, Trichopoulos D, Jensen OM. Birth order and risk of testicular cancer. *Cancer Causes Control* 1992;3:265-72.
- (27) Moller H, Skakkebaek NE. Testicular cancer and cryptorchidism in relation to prenatal factors: case-control studies in Denmark. *Cancer Causes Control* 1997;8:904-12.
- (28) Swerdlow AJ, Huttly SR, Smith PG. Prenatal and familial associations of testicular cancer. *Br J Cancer* 1987;55:571-7.
- (29) Lanier AP, Noller KL, Decker DG, Elveback LR, Kurland LT. Cancer and stilbestrol. A fol-

- low-up of 1,719 persons exposed to estrogens *in utero* and born between 1943–1959. *Mayo Clin Proc* 1973;48:793–9.
- (30) Dieckmann WJ, Davis ME, Rynkiewicz LM, Pottinger RE. Does the administration of diethylstilbestrol during pregnancy have therapeutic value? *Am J Obstet Gynecol* 1953;66:1062–81.
 - (31) Breslow N, Day N. Statistical methods in cancer research. Volume II—the design and analysis of cohort studies. IARC Scientific Publication 88. Lyon (France): IARC Sci Publ; 1987.
 - (32) SAS/STAT software: changes and enhancements through release 6.11. Cary (NC): SAS Institute, Inc.; 1996.
 - (33) StatXact 4 for Windows, statistical software for exact nonparametric inference. Cambridge (MA): Cytel Software Corporation; 1999.
 - (34) Smith O, Smith G. Use of diethylstilbestrol to prevent fetal loss from complications of late pregnancy. *N Engl J Med* 1949;241:562–8.
 - (35) Greco TL, Furlow JD, Duello TM, Gorski J. Immunodetection of estrogen receptors in fetal and neonatal mouse reproductive tracts. *Endocrinology* 1992;130:421–9.
 - (36) Raipert-De Meyts E, Skakkebaek NE. The possible role of sex hormones in the development of testicular cancer. *Eur Urol* 1993;23:54–9; discussion 60–1.
 - (37) Wilson JD, George FW, Griffen JE. The hormonal control of sexual development. *Science* 1981;211:1278–84.
 - (38) Newbold RR, Bullock BC, McLachlan JA. Mullerian remnants of mice exposed prenatally to diethylstilbestrol. *Teratog Carcinog Mutagen* 1987;7:377–89.
 - (39) Clark JH, Hsueh AJ, Peck EJ. Regulation of estrogen receptor replenishment by progesterone. *Ann N Y Acad Sci* 1977;286:161–79.
 - (40) Hsueh AJ, Peck EJ, Clark JH. Progesterone antagonism of the oestrogen receptor and oestrogen-induced uterine growth. *Nature* 1975;254:337–9.
 - (41) Tseng L, Gurside E. Effects of progestins on estradiol receptor levels in human endometrium. *J Clin Endocrinol Metab* 1975;41:402–4.
 - (42) Wittliff J. Steroid hormone receptors in breast cancer. *Cancer* 1984;53:630–43.
 - (43) vom Saal F, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, et al. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. *Proc Natl Acad Sci USA* 1997;94:2056–61.
 - (44) Zheng T, Holford TR, Ma Z, Ward BA, Flannery J, Boyle P. Continuing increase in incidence of germ-cell testis cancer in young adults: experience from Connecticut, USA, 1935–1992. *Int J Cancer* 1996;65:723–9.

NOTES

¹*Editor's note:* SEER is a set of geographically defined, population-based, central cancer registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Registry data are submitted electronically without personal identifiers to the NCI on a biannual basis, and the NCI makes the data available to the public for scientific research.

Supported by Public Health Service contract N01CP21167 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

We thank study coordinators Ann M. Lalone and Mary Ziegler for invaluable assistance with participant and specimen tracing; study coordinators Shafika Abrahams-Gessel, Charise Cowan, Diane Anderson, and Kathleen Rowlings for participant follow-up; Patricia Cody from DES Action and Dr. Susan Helmrich and Margaret Lee Braun from the DES Cancer Network for support; Winifred Ricker for data analysis; and the staff at Westat, Inc. (Rockville, MD), including Bob Saal and Peter Sebeny, for study-wide coordination efforts. Finally we thank the diethylstilbestrol-exposed and unexposed men for their participation in this study.

Manuscript received May 22, 2000; revised January 22, 2001; accepted January 29, 2001.